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PATENT

Attorney Reference Number 6395-61708  
Application Number 10/009,660

**Amendments to the Specification**

Please replace the paragraph beginning at page 9, line 20, with the following amended paragraph:

In one embodiment of the present invention, the fluorescently labeled antigens comprise intact bacteria that are directly attached to fluorescent molecules. The fluorescent molecules may include, but are not limited to, fluorescein and its derivatives such as 5, 6, carboxyfluorescein succinimidyl ester, ethidium monoazide (EMA), phycoerythrin, allophycocyanin, phycocyanin, rhodamine, tetramethylrhodamine, Texas-Red TEXAS RED (sulforhodamine-101-sulfonyl chloride), EDANS (5-((2-aminoethyl)amino)naphthalene-1-sulfonic acid, sodium salt), BODIPY (borondipyrromethene difluoride) dyes, Cy3 and Cy5. The present invention provides methods for an opsonophagocytic assay wherein one or more of fluorescent molecules are used to label, and thereby distinguish, different serotypes of a pathogenic species. The binding of functional antibodies in a biological sample to the differently labeled antigens results in the attachment of a different label to each functional antibody specific for a given serotype antigen. The labels may be differentiated by differences in their emission spectra and/or differences in their fluorescence intensity. For example, in one embodiment of the present invention, functional antibodies specific for four different serotypes of a specific pathogen are simultaneously detected and distinguished, preferably in a flow cytometer, by labeling the antigens bound by those antibodies with different fluorescent colors. Alternatively, the antigens are labeled with the same fluorescent color, but with different fluorescent intensities that are distinguished, preferably in a flow cytometer. As a third alternative embodiment, the four antigens are labeled with two fluorescent colors and the two antigens labeled with the same color are labeled with different fluorescent intensities. As a fourth alternative, the calculated ratio of emissions of two or more fluorescent dyes may be used to distinguish individual bacteria or bead populations.

Please replace the paragraph beginning at page 11, line 17, with the following amended paragraph:

In an alternate embodiment of the present invention, the fluorescently labeled antigens comprise fluorescent beads attached to or coated with polysaccharide antigens. The fluorescent

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beads may include, but are not limited to, beads containing fluorescein and its derivatives such as 5, 6, carboxyfluorescein, ethidium monoazide (EMA), phycoerythrin, allo-phycoyanin, phycocyanin, rhodamine, tetramethylrhodamine, Texas-Red TEXAS RED (sulforhodamine-101-sulfonyl chloride), EDANS (5-((2-aminoethyl)amino)naphthalene-1-sulfonic acid, sodium salt), BODIPY (borondipyrromethene difluoride) dyes, Cy3 and Cy5. In a preferred embodiment, the fluorescent beads are coated with antigens by modification of polysaccharide antigens by the addition of an aldehyde group and modification of the beads by the addition of carboxy surface groups as described in more detail below. It is to be understood that the present invention is not limited by the type of bead used, as beads useful for attachment of antigens are known to those skilled in the art, and any structure that is capable of binding antigen and functioning as the described beads herein is encompassed by the present invention. The fluorescent beads may be, but are not limited to, latex beads, polystyrene beads and magnetic beads, however polystyrene beads are preferred. While not wishing to be limited, one example of beads suitable for use in the present invention is described in Example 8.